Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.14.6163

World J Gastroenterol 2008 October 28; 14(40): 6163-6170
World Journal of Gastroenterology ISSN 1007-9327

Ataxia telangiectasia-mutated-Rad3-related DNA damage checkpoint signaling pathway triggered by hepatitis B virus infection

Fan Zhao, Ning-Bo Hou, Xiao-Li Yang, Xiang He, Yu Liu, Yan-Hong Zhang, Cong-Wen Wei, Ting Song, Li Li, Qing-Jun Ma, Hui Zhong

Fan Zhao, Ning-Bo Hou, Xiang He, Yan-Hong Zhang, Cong-Wen Wei, Ting Song, Li Li, Qing-Jun Ma, Hui Zhong, Molecular Genetics Department, Beijing Institute of Biotechnology, Beijing 100850, China
Xiao-Li Yang, Yu Liu, Biochemical and Immunology Analyze Center, The General Hospital of Chinese People's Armed Police Forces, Beijing 100039, China
Author contributions: Zhao F and Hou NB contributed equally to this work; Ma QJ, Li L, Zhang YH and Zhong H designed research; Yang XL, He X, Liu Y, Zhang YH, Wei CW, Song T and Li L performed research; Zhong H wrote the paper.
Supported by National Natural Science Foundation of China, No. 30700413
Correspondence to: Hui Zhong, PhD, Molecular Genetics Department Beijing Institute of Biotechnology, Beijing 100850, China. towall@yahoo.com
Telephone: +86-10-66931809 Fax: +86-10-88272563
Received: April 14, 2008 Revised: June 21, 2008
Accepted: June 28, 2008
Published online: October 28, 2008

Abstract

AIM: To explore whether acute cellular DNA damage response is induced upon hepatitis B virus (HBV) infection and the effects of the HBV infection.

METHODS: We incubated HL7702 hepatocytes with HBV-positive serum, mimicking a natural HBV infection process. We used immunoblotting to evaluate protein expression levels in HBV-infected cells or in non-infected cells; immunofluorescence to show ATR foci ands Chk1 phosphorylation foci formation; flow cytometry to analyze the cell cycle and apoptosis; ultraviolet (UV) radiation and ionizing radiation (IR)-treated cells to mimic DNA damage; and Trypan blue staining to count the viable cells.

RESULTS: We found that HBV infection induced an increased steady state of ATR protein and increased phosphorylation of multiple downstream targets including Chk1, p53 and H2AX. In contrast to ATR and its target, the phosphorylated form of ATM at Ser-1981 and its downstream substrate Chk2 phosphorylation at Thr-68 did not visibly increase upon infection. However, the level of Mre11 and p21 were reduced beginning at 0.5 h after HBV-positive serum addition. Also, HBV infection led to transient cell cycle arrest in the S and the G2 phases without accompanying increased apoptosis. Research on cell survival changes upon radiation following HBV infection showed that survival of UV-treated host cells was greatly increased by HBV infection, owing to the reduced apoptosis. Meanwhile, survival of IR-treated host cells was reduced by HBV infection.

CONCLUSION: HBV infection activates ATR DNA damage response to replication stress and abrogates the checkpoint signaling controlled by DNA damage response.

Key words: Hepatitis B virus; DNA damage response; Cell cycle; p21; Mre11

Peer reviewer: Dr. Michael Torbenson, Department of Pathology, Johns Hopkins University School of Medicine, 1503 E Jefferson, Room B314, Baltimore 21231, United States

Zhao F, Hou NB, Yang XL, He X, Liu Y, Zhang YH, Wei CW, Song T, Li L, Ma QJ, Zhong H. Ataxia telangiectasia-mutated-Rad3-related DNA damage checkpoint signaling pathway triggered by hepatitis B virus infection. *World J Gastroenterol* 2008; 14(40): 6163-6170 Available from: URL: http://www.wjgnet.com/1007-9327/14/6163.asp DOI: http://dx.doi.org/10.3748/wjg.14.6163

INTRODUCTION

Eukaryotic cells employ multiple strategies of checkpoint signaling and DNA repair mechanisms to monitor and repair damaged DNA[1-5]. There are two branches of the checkpoint response pathway, ataxia telangiectasia-mutated (ATM) pathway and ATM-Rad3-related (ATR) pathway. The major difference between ATM and ATR is the type of DNA damage to which each responds. For example, ATM responds to ionizing radiation (IR) and other agents that cause double-strand breaks (DSBs) in DNA. ATR responds to ultraviolet radiation (UV) radiation and other agents that induce the accumulation of stalled replication forks and subsequent single-stranded breaks (SSBs) in DNA. The DSBs are recognized by the Mre11-Rad50-Nbs1 complex, which recruits and activates ATM kinase[6]. The SSBs are coated

by replication protein A, and it recruits a complex of ATR kinases and ATR-interacting protein, which is then activated by the Rad9-Rad1-Hus1 complex and other factors. Accumulating evidence suggests that checkpoint signaling through ATR is intimately linked to the process of DNA replication[7,8].

A variety of checkpoint and DNA repair proteins have been identified as substrates for ATM and ATR kinases, including the checkpoint kinases Chk1 and Chk2, as well as p53, Smc1 and H2AX. Chk1 was first identified in *Schizosaccharomyces pombe* because of its role in the checkpoint arrest at G2/M, and it is mainly phosphorylated by ATR in response to UV, hydroxyurea and aphidicolin[9-11]. p21 was initially identified as a component of quaternary complex containing cdk-cyclin kinases and PCNA. Previous study has shown that p21 protein is degraded after low doses of UV; this degradation is essential for optimal DNA repair and is ATR-dependent[12]. The MRN complex consisting of Mre11, Rad50, and Nbs1 is a target of both ATM and ATR and is involved in both pathways[13-15].

Virus replication presents the host cells with large amounts of exogenous genetic material, including DNA ends and unusual structures. Therefore, infected cells recognize viral replication as a DNA damage stress and elicit DNA damage signal transduction, which ultimately induces apoptosis as part of host immune surveillance. However, recent reports have shown that viruses evolve a variety of mechanisms to manipulate DNA damage signaling for their replication and propagation. For example, Epstein-Barr Virus (EBV)[16] abrogates the p53 checkpoint signaling pathway through the interaction of the BZLF1 protein and p53 to avoid apoptosis. Other viruses such as human immunodeficiency virus type 1 (HIV-1)[17-20], herpes simplex virus type 1 (HSV-1)[21,22] and human cytomegalovirus[23] can activate and exploit a cellular DNA damage response, which aids viral replication. Adenovirus blocks ATM signaling and concatemer formation through targeting the DNA repair complex of MRN for degradation and mislocalization[24,25]. Thus, under some circumstances, viruses have co-opted endogenous checkpoint regulators to ensure their own efficient replication[26,27].

Hepatitis B virus (HBV) is a partially enveloped double-stranded DNA virus with a genome of 3.2 kb. Upon infection, the viral genome is transported into the cell, where it is converted into a covalently closed circular DNA (cccDNA). The cccDNA serves as a template for transcription by host cell RNA polymerase Ⅱ. The pregenomic RNA is then reverse transcribed into DNA replicative intermediates in the cytoplasm within immature viral core particles, by the virally encoded polymerase. Integration into host chromosome may happen during its replication.

There is no evidence thus far that the ATM/ATR kinases or their downstream pathways are triggered by HBV infection. The present study was undertaken by culturing normal hepatocyte cell line HL7702 and primary hepatocytes from a healthy liver donor with HBV-positive serum, mimicking natural HBV infection. We showed here, perhaps for the first time, that HBV infection elicited acute cellular DNA damage response dependent on ATR. However, the ATR checkpoint signaling was blocked downstream of p53-dependent and p53-independent pathways to evade apoptosis.

MATERIALS AND METHODS

Chemicals

Mimosine and aphidicolin were obtained from Sigma. The stock concentration of mimosine was 100 mmol/L, the stock concentration of aphidicolin was 10 mmol/L; both were dissolved in Dimethyl Sulphoxide (DMSO).

Cell culture, synchronization and infection

The human hepatocyte cell line HL7702, which was isolated from a HBV-seronegative individual, was obtained from Shanghai Biochemistry Institute. HL7702 were cultured in RPMI-1640 with 10% heat-inactivated FBS (Gibco). Serum samples from HBV carriers were analyzed. The patient was anti-HBsAg-positive, as detected by ELISA (SIIC Ke-Hua, Shanghai), and HBV DNA in the serum sample was quantified using FQ-PCR (Da-An Gene Corp). The patient had received no antiviral therapy prior to the study and was not infected with HCV or HIV. The number of serum HBV viral particles was 7×10^9 copies/mL, as quantified by FQ-PCR. Normal serum was obtained from healthy non-infected individuals as a control. The sera were stored at -80℃ until use.

When synchronized, the HL7702 cells were cultured in RPMI-1640 containing 0.1% FBS for 2 d; the culture medium was then replaced with fresh RPMI-1640 including 10% FBS and 200 μmol/L mimosine for 24 h, in order to arrest the cells at G1-S phase. The arrested cells were then washed twice, and the culture medium was replaced with RPMI-1640 containing normal or HBV-positive serum. The cells were then harvested at different times after mimosine release. All procedures were performed under level P2 biosafety conditions to minimize the possibility of cross-contamination.

Primary culture of human hepatocytes

Hepatocytes were prepared from a 35-year-old healthy male liver donor according to previously described procedures[28]. Briefly, the liver tissues were cut with scissors into 0.1-0.5 mm^3 pieces and were shattered with a 5-mL syringe into single cells or cell aggregates. Cells were seeded into 12-well culture dishes and incubated with 1 mL of 10% FBS in RPMI-1640 at 37℃ under 50 mL/L CO_2. The medium was changed after the first 48 h with serum-free medium. The serum-free medium was composed of DMEM/F12 (1:1) and 0.01 nmol/L nicotinamide, 0.02 ng/L epidermal growth factor (EGF), 0.02 ng/L basic fibroblast growth factor (bFGF), 0.365 ng/L glutamate, B27 (1:50, Sigma), 0.1 U/L penicillin, 0.1 ng/L streptomycin, and 0.1 ng/L fluconazole. HBV-positive serum was added to the culture medium 2 wk after hepatocyte phenotype cell development, and cells were harvested for immunoblotting assay 3 h later.

Immunoblotting assay

Cell extracts were lysed in ice-cold Tris buffer (50 mmol/L, pH 7.5) containing 5 mmol/L EDTA, 300 mmol/L NaCl, 0.1% Igepal, 0.5 mmol/L NaF, 0.5 mmol/L Na_3VO_4, 0.5 mmol/L PMSF, and antiprotease mixture (Roche Molecular Biochemicals) for 30 min on ice and centrifuged at 13 000 *g* for 10 min. The supernatant protein concentration was determined by the Bradford procedure (BioRad). The proteins were resolved on 15% SDS-PAGE and transferred onto nitrocellulose membranes. Blots were blocked in TBST containing 5% non-fat dried milk (NFDM) and incubated with primary antibodies as follows: antibodies against p21, Mre11, ATR, (Santa Cruz) and tubulin (Sigma) were incubated at room temperature for 1 h. Antibodies against ATM phosphoserine 1981 (ATMp), Chk2 phosphothreonine 68 (Chk2p), Chk1 phosphoserine 345 (Chk1p), p53 phosphoserine 15 (p53p) and H2AX phosphoserine 139 (H2AXp) (Cell Signaling) were incubated at 4℃ overnight. Secondary antibodies were from Jackson Laboratories. Horseradish-peroxidase-based detection was performed using a chemiluminescence reagent (Amersham Biosciences), according to the manufacturer's instructions.

DNA damage sensitivity assays

For radiation sensitivity assays, cells were irradiated with the indicated doses of γ rays from a ^{137}Cs source for indicated times, or 254 nm UV light with complete medium plated in triplicate. HBV-positive serum was added to the cultures before indicated doses of UV or IR radiation. Cells were washed extensively to remove viral inputs 24 h after HBV-positive serum addition, and then treated with different dose of UV or IR radiation. After 48 h of UV and 4 d of IR treatment, cells were collected and surviving cells were counted with Trypan blue staining. The percentage survival was determined by quantization of the relative viable number of treated cells divided by the viable number of untreated cells.

Flow cytometry

For propidium iodide staining, cells were harvested by trypsinization, fixed with ice-cold 70% ethanol, and resuspended in a solution containing 50 mg/L propidium iodide, 0.1% Triton X-100, 50 mg/L RNase A, and 5 mmol/L EDTA at room temperature (RT) for 1 h. Cells were then diluted 1:1 in 1% BSA PBS for cytometric analysis.

Immunofluorescence assay

HL7702 cells were grown on glass coverslips for 12 to 18 h prior to infection. At 0.5 h post-infection, cells were washed briefly in phosphate buffered saline (PBS) and fixed in 4% paraformaldehyde (PFA) in PBS for 10 min, then permeabilized for 10 min in 1% Triton X-100 in PBS. For the visualization of detergent-resistant or chromatin-associated nuclear proteins, an *in situ* extraction method that removed the cytoplasm and nucleosolic proteins was used. Cells were pre-extracted for 5 min on ice with 0.5% Triton X-100 in cytoskeletal buffer as described previously[29] then fixed in 4% PFA for 10 min. After washing with PBS and blocking in 2% fetal bovine serum (FBS) in PBS, cells were incubated with primary antibodies diluted in 2% FBS overnight at 4℃. Anti-Chk1p was used at a concentration of 1:100, while anti-ATR was used at 1:400. After washing with PBS three times, cells were incubated with secondary antibodies diluted 1:200 in 2% FBS for 30 min, then washed again three times with PBS. Next, the nuclei were stained with DAPI diluted in PBS for 10 min. After a final wash in PBS, samples were preserved in glycerol and images were captured by using a Zeiss LSM510 confocal microscope.

RESULTS

HBV infection induced a cellular DNA damage response dependent on ATR

To explore whether acute cellular DNA damage response was induced upon HBV infection, we incubated HL7702 hepatocytes with HBV-positive serum to examine the phosphorylation status of DNA damage response proteins. 10^5 HL7702 monolayer cells in a 6-cm plate were infected with HBV-positive serum containing 10^6 HBV at 37℃ under 50 mL/L CO_2. Serum from uninfected individuals was used as a non-infected control. Cells were washed eight times to remove excess viral inputs before harvesting. Whole-cell lysates from the HBV-infected and non-infected cultures were examined for the status of the DNA damage response proteins. Figure 1A reveals that HBV infection induced an increase in the steady state levels of the ATR protein and in the phosphorylation levels of its downstream substrates Chk1, p53 and H2AX. An increase in Chk1 phosphorylation at Ser-345 was evident at 3 h from the start of HBV-positive serum addition, with further increase from 6 h of infection (hoi) to 48 hoi. The phosphorylation of p53 Ser-15 was elevated beginning at 24 hoi and increased greatly at 48 hoi. Finally, there was a sharp increase in the amount of phosphorylated H2AX Ser-139 beginning at 24 hoi. To confirm these results, primary hepatocytes from a healthy male liver donor were obtained and incubated with HBV-positive serum for 3 h; obvious Chk1 phosphorylation was detected in HBV-infected cells (Figure 1B). We then used immunofluorescence to examine the localization of these proteins early after infection. Figure 1C shows that ATR foci as well as the Chk1 phosphorylation foci in non-infected cells were very faint, as opposed to the HBV infected cells where the foci were larger, more numerous and much brighter.

In contrast to ATR and its targets, the phosphorylated form of ATM at Ser-1981 was not visibly increased upon infection, and phosphorylation of its downstream substrate Chk2 at Thr-68 began to decrease starting from 3 hoi (Figure 1A), with slight recovery at later time points, indicating that the p53-independent pathway was blocked. Phosphorylation of p53 at Ser-15 in response to DNA damage usually correlates with the ability of p53 to trans-activate downstream target genes. Therefore, we examined expression levels of p53 transcriptional targets $p21^{cip1/waf1}$, a cyclin-dependent kinase inhibitory protein. Figure 1A

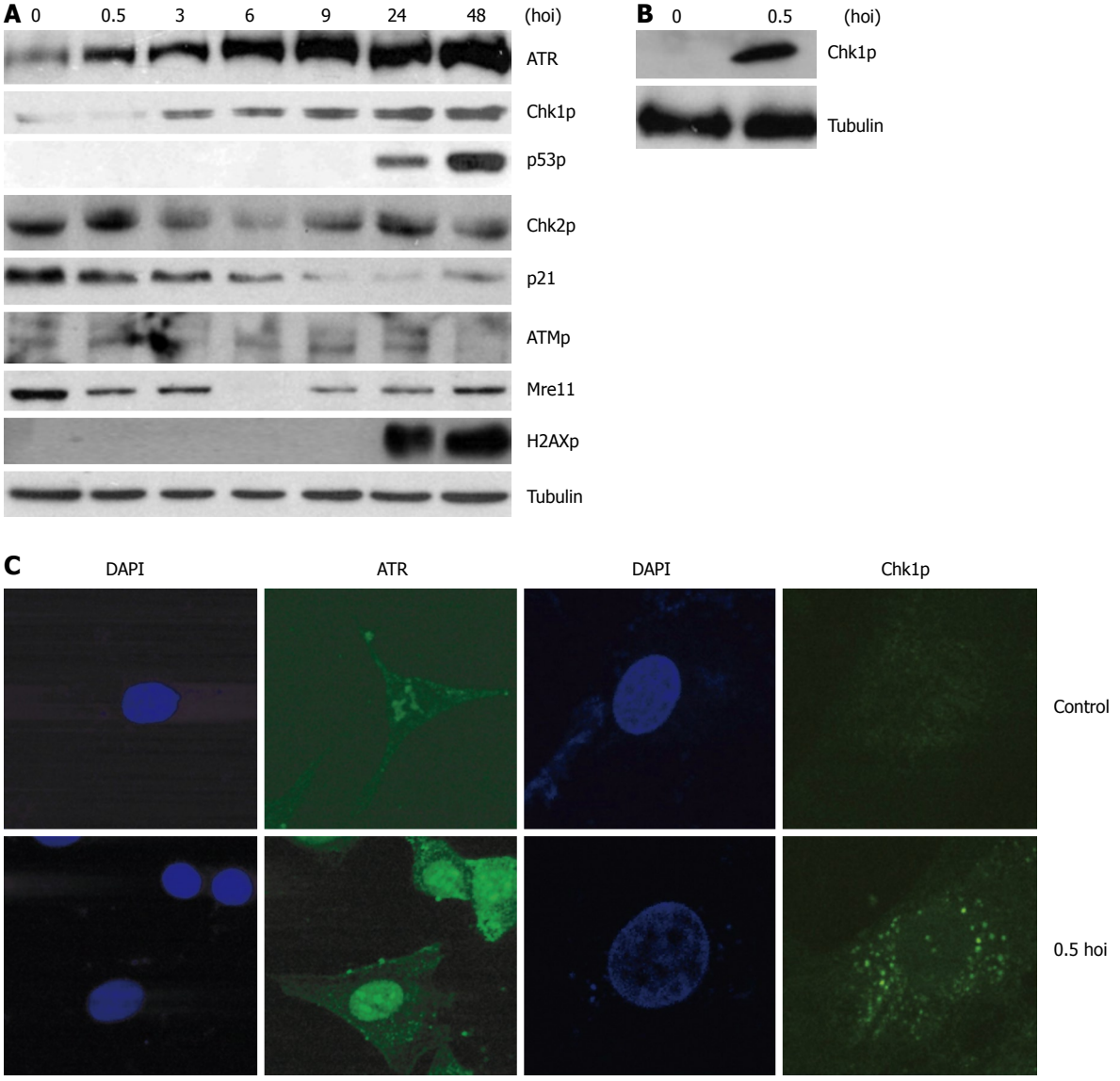

Figure 1 HBV infection activates a cellular checkpoint response dependent on ATR. A: 105 human hepatocyte HL7702 monolayer cells in a 6-cm plate were infected with 106 virus particles from HBV-positive patients at 37℃ under 50 mL/L CO_2; normal serum from healthy individuals was used as a non-infected control. Prior to cell harvesting, the cells were washed eight times thoroughly to remove excess viral input. Whole-cell lysates were prepared at various times of infection (hoi) and subjected to an immunoblotting assay by using antibodies against the indicated proteins. Tubulin was used as the equal loading control; B: Primarily cultured hepatocyte cells were prepared and were harvested 3 h after HBV-positive serum addition. Whole cell lysates were prepared and subjected to immunoblotting assay by using Chk1 phosphorylation antibody; tubulin was used as the equal loading control; C: HL7702 cells were infected with HBV-positive serum for 0.5 h. Normal serum from healthy individuals was used as a non-infected control. Immunofluorescence with antibodies to Chk1 Ser-345 and ATR (green) were monitored. DNA was stained with DAPI (blue).

shows that the amount of p21 decreased substantially with time after infection, suggesting that p53-dependent downstream signaling was blocked during HBV infection, despite the appearance of phosphorylated p53. By 0.5 h after HBV-positive serum addition, downregulation of Mre11 began to be detected. This reduction in Mre11 protein occurs shortly after HBV infection, implying that an incoming virion protein may lead to this degradation. Further investigation is required to explain this phenomenon.

Since HBV infection activated DNA damage checkpoint pathway that responded to replication stress, we asked if HBV infection would effect cell cycle progression and cell death. Cells were synchronized in G1 phase by mimosine, an inhibitor of DNA synthesis initiation, for 24 h. Arrested cells were then washed twice and media replaced with RPMI-1640 containing HBV serum or normal serum. Cells were harvested at different times after mimosine release. The results of fluorescence-activated cell sorting (FACS) analysis showed that approximately 5% of the cells were in early S phase with 95% in G1 after synchronization (Figure 2A), indicating that the synchronization was fulfilled. Control cells treated with normal serum had approximately 44.4% of cells in S phase after 12 h release from mimosine, whereas cells treated with HBV-positive serum still had 56.6% of cells in S phase (Figure 2A and B). The percentage of total cells in S phase indicates the effect of virus in extending the period of DNA synthesis. Toward the end of the cell cycle, there was an accumulation of cells

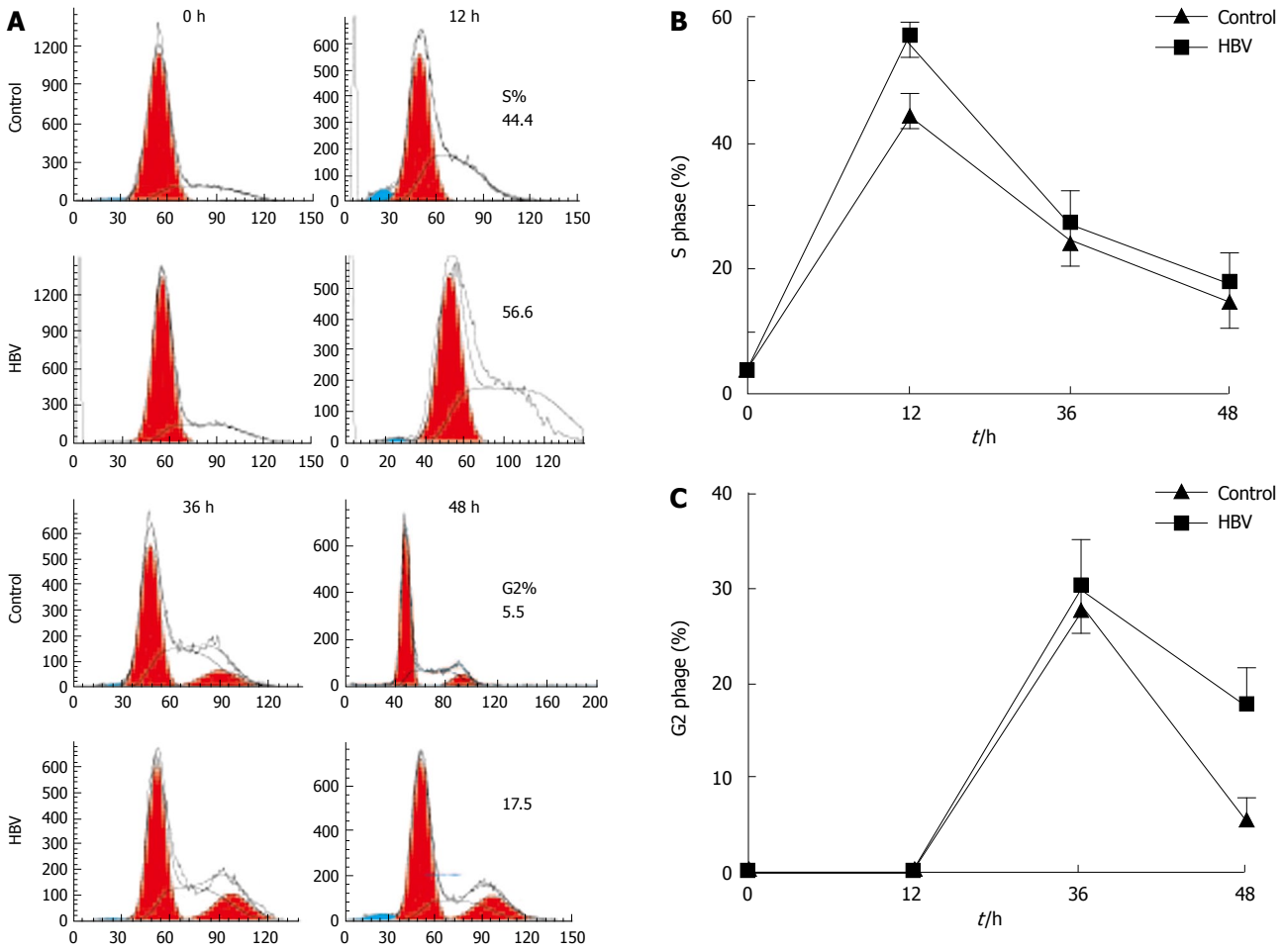

Figure 2 HBV infection caused transient cell cycle arrest in the S and the G2 phase. The duration of the S and G2 phase were measured in HBV-infected or non-infected cells. Cells were synchronized with mimosine for 24 h, and then media were replaced with RPMI-1640 containing 10% HBV serum or normal serum. At indicated times, cells were harvested and examined for cell cycle profile using propidium iodide staining and flow cytometry. A: Cell cycle profile in HBV-infected cells and in non-infected cells; B: Percentages of cells in the S phase; C: Percentages of cells in the G2 phase. Mean and standard error are presented for three independent experiments.

in G2 phase in HBV-infected cells (5.5% for control cells versus 17.5% for HBV infection cells) without the appearance of fragments with less than 2N DNA (Figure 2A and C). These findings indicate that DNA damage pathway responding to replication stress induced by HBV infection will lead to transient cell cycle arrest without accompanying increased apoptosis.

Checkpoint signaling pathway was compromised in HBV-infected cells

Previous results show that HBV infection did not show increased apoptosis even though a cellular DNA damage response dependent on ATR was activated (Figure 2A). To better understand the consequences of the ATR signaling pathway triggered by HBV infection, we examined if HBV infection had an impact on host cell survival after IR and UV radiation. Cells were washed extensively to remove viral inputs 24 h after HBV-positive serum addition and then treated with different doses of UV or IR radiation. After 48 h of UV and 4 d of IR treatment, cells were collected and surviving cells were counted with Trypan blue staining. Figure 3A and B showed that the survival rate of UV-irradiated cells was enhanced by HBV infection, while the survival rate of IR-irradiated cells was reduced by HBV infection. Figure 3C shows that while the cell survival rate after 8 s of UV or 4 Gy of IR was about 30%, the survival rate of HBV-infected cells after 8 s of UV radiation increased from 30% to 49%. By contrast, HBV infection pre-treatment with 4 Gy of IR radiation reduced the cell survival rate from 31% to 21%, suggesting that survival of UV-treated cells was enhanced by HBV infection (Figure 3C). Based on this result, we analyzed the difference in apoptosis between UV radiation only and combined treatment of UV with HBV infection. Decreased apoptosis was seen in combined treatment cells (Figure 4A and B). Twenty-four percent apoptosis was seen in UV-radiation-only cells, while about 8% apoptosis was detected in cells treated with UV radiation followed by HBV infection. It is known that cell cycle checkpoint triggered by DNA damage response induces cell apoptosis if damaged DNA cannot be repaired. The inhibitory effect of HBV on cell survival was reduced in cells treated with UV, indicating that checkpoint signaling controlled by the ATR DNA damage pathway was at least partially compromised in HBV-infected cells; therefore, the ATR signal pathway did not function completely.

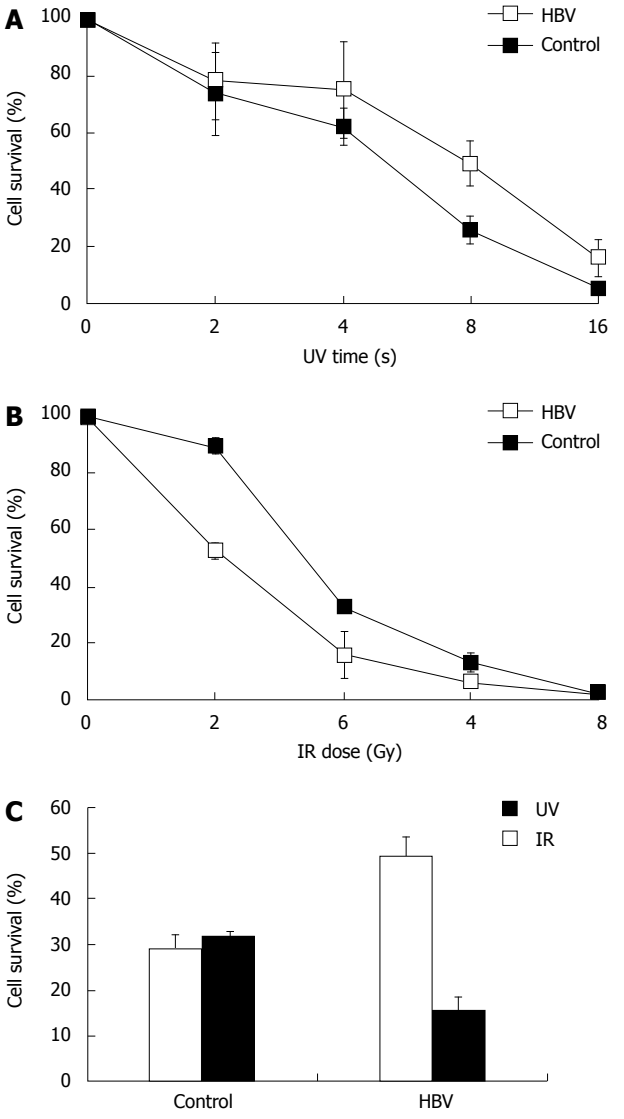

Figure 3 HBV infection hypersensitizes host cells to IR and causes hyper-resistance of host cells to UV. A: HL7702 cells were treated with indicated doses of IR followed HBV positive serum addition for 24 h, and continued in culture for another 4 d, trypan blue staining was used for viable cell counting; B: HL7702 cells were treated with indicated doses of UV followed by HBV-positive serum addition for 24 h, and continued culture for another 48 h, trypan blue staining was used for viable cell counting; C: HL7702 cells were treated with 8 s of UV or 4 Gy of IR followed HBV infection, and viable cells were counted. Percentage survival was determined by the number of treated cells normalized to untreated cells. Mean and standard error are presented for three independent experiments.

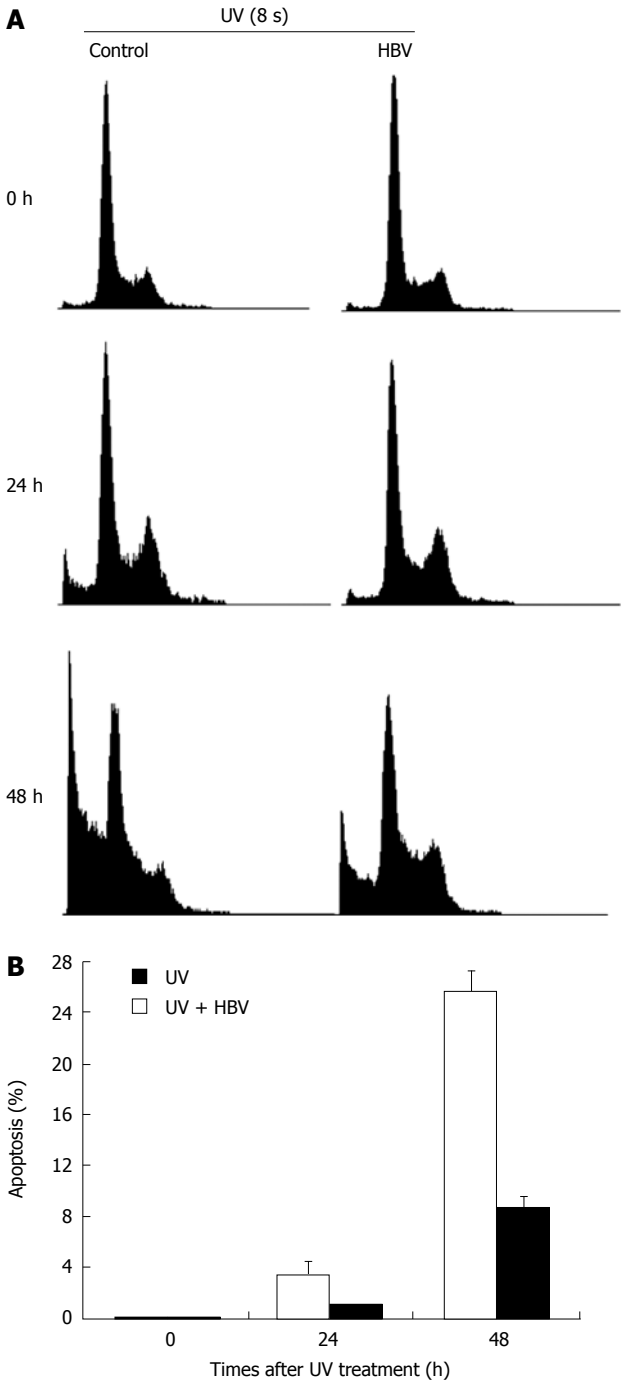

Figure 4 HBV infection followed by UV radiation led to decreased cell apoptosis. A: HBV-positive serum was added to the culture medium before UV radiation treatment. Cells were washed extensively 24 h after HBV-positive serum addition and then treated with 8 s of UV radiation. Cell cycle profile was examined by propidium iodide staining and flow cytometry at indicated times after UV treatment; B: Apoptosis percentage after UV radiation followed HBV infection. Mean and standard error are presented for three independent experiments.

DISCUSSION

The induction of cell cycle checkpoints and activation of the ATM/ATR-dependent pathway have been reported to accompany infection by a number of different viruses. Recent reports have shown that viruses have evolved a variety of mechanisms to manipulate DNA damage signaling for their replication and propagation. In this study, HL7702 cells and primarily cultured hepatocytes were inoculated with HBV-positive serum (10^6 particles per 10^5 cells), mimicking the HBV infection process. Serum from healthy individuals was used as a non-infected control. We propose that HBV infection induces acute cellular DNA damage response dependent on ATR, as demonstrated by ATR protein and increased phosphorylation of Chk1, p53 and H2AX. Since Chk1 phosphorylation, ATR foci formation, and Mre11 and p21 degradation happened shortly after HBV-positive serum addition, we propose that incoming virion protein and genetic materials triggered this response. p53 and

H2AX phosphorylation did not begin to accumulate until 24 h after HBV serum addition, implying HBV replication inside the infected cells may be responsible for these phenomena. Interesting questions are raised and need further investigation.

Although ATM/ATR kinase phosphorylates Chk2 at Thr-68 and p53 at Ser-15, the ATR kinase predominantly targets Chk1 at Ser-345, leading to increased Chk1 activity. Our results indicate that HBV infection preferentially activates ATR DNA damage response signaling, as is the case with human adeno-associated-virus type 2[30-36].

As a latent virus, HBV abrogates checkpoint signaling controlled by ATR, to prevent triggering of signals for apoptosis in multiple ways. The mechanism of regulation of apoptosis by HBV was *via* both p53-dependent and p53-independent pathways. p53-dependent cell cycle checkpoint features p21-mediated inactivation of cdk2/cyclinE; HBV abrogates p53 dependent checkpoint activation by p21 degradation. Chk2 inhibition inhibits cdk2/cyclin E activity by phosphorylation of cdk2 at Tyr-15 in a p53-independent fashion, and the virus-decreased phosphorylation of Chk2 by Mre11 degradation inhibits the p53-independent DNA damage signaling pathway. It is known that ATM and MRN complex function in a common pathway, and the MRN complex can function to activate ATM kinase activity, so degradation of Mre11 protein by virus would inhibit ATM kinase activity, and thus affect phosphorylation of its downstream target Chk2. Therefore, like adenovirus[24,25], HBV appears to have evolved double check mechanisms to block cell cycle checkpoint signaling pathways. Consistent with this, HBV has been reported to express an additional anti-apoptotic gene HBV X that mislocates p53 to the cytoplasm, in order to evade host cellular DNA damage response and modulate apoptosis. HBX has also been reported to sensitize liver cells to environmental carcinogens, including diethylnitrosamine and aflatoxin B and UV. We observed that HBV virus enhanced cell survival upon UV radiation but hypersensitized host cells to IR; this discrepancy may be due to the complex interactions between the virus as a whole and DNA repair machinery, indicating that the virus mainly blocks cellular signaling checkpoint dependent on ATR. The risk of acquiring mutations would be enhanced by compromised cellular DNA repair caused by HBV infection. Accordingly, exposure to other environmental risk factors should act synergistically to favor the carcinogenesis process[37-39].

In summary, HBV induces cellular DNA damage response dependent on ATR, but escapes the consequences of activation of the DNA damage checkpoint by degradation of checkpoint proteins at different levels. The implication of this is that with time, persistent HBV infection may lead to the accumulation of a variety of mutations which would ultimately give rise to hepatocellular carcinoma.

COMMENTS

Background

Eukaryotic cells employ multiple strategies of checkpoint signaling and DNA repair mechanisms to monitor and repair damaged DNA. There are two branches of the checkpoint response pathway, ataxia telangiectasia-mutated (ATM) pathway and ATM-Rad3-related (ATR) pathway. Virus replication presents the host cells with large amounts of exogenous genetic material, including DNA ends and unusual structures. Thus, infected cells recognize viral replication as a DNA damage stress and elicit DNA damage signal transduction, which ultimately induces apoptosis as part of host immune surveillance. However, recent reports have shown that viruses evolve a variety of mechanisms to manipulate DNA damage signaling for their replication and propagation.

Research frontiers

Epstein-Barr virus (EBV) abrogates p53 checkpoint signaling pathway through the interaction of the BZLF1 protein and p53 to avoid apoptosis. Other viruses such as human immunodeficiency virus type 1 (HIV-1), herpes simplex virus type 1 (HSV-1) and human cytomegalovirus can activate and exploit a cellular DNA damage response, which aids viral replication. Adenovirus blocks ATM signaling and concatemer formation through targeting the DNA repair complex of MRN for degradation and mislocalization. Thus, under some circumstances, viruses have co-opted endogenous checkpoint regulators to ensure their own efficient replication.

Innovations and breakthroughs

There was no evidence indicating that the ATM/ATR kinase or their downstream pathways were triggered by hepatitis B virus (HBV) infection. The present study was undertaken by culturing normal hepatocyte cell line HL7702 and primary hepatocytes from a healthy liver donor with HBV-positive serum, mimicking natural HBV infection. We showed, perhaps for the first time, that HBV infection elicited acute cellular DNA damage response dependent on ATR. However, the ATR checkpoint signaling was blocked downstream of p53-dependent and p53-independent pathways to evade apoptosis.

Applications

Since DNA damage response is an acute response that happens quickly after virus infection, we assume that early intervention of the DNA damage pathway will function more efficiently and can be used clinically as HBV infection therapy during its early infectious stage or fulminant HBV infection.

Peer review

In this interesting study the authors investigated whether exposure to HBV infection will upregulate DNA damage checkpoint signaling pathways. They show that ATM-Rad3 is upregulated as well as several downstream targets.

REFERENCES

1 **Shiloh Y**. ATM and related protein kinases: safeguarding genome integrity. *Nat Rev Cancer* 2003; **3**: 155-168
2 **Sancar A**, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu Rev Biochem* 2004; **73**: 39-85
3 **Bakkenist CJ**, Kastan MB. Initiating cellular stress responses. *Cell* 2004; **118**: 9-17
4 **Lim DS**, Kim ST, Xu B, Maser RS, Lin J, Petrini JH, Kastan MB. ATM phosphorylates p95/nbs1 in an S-phase checkpoint pathway. *Nature* 2000; **404**: 613-617
5 **Brown EJ**, Baltimore D. Essential and dispensable roles of ATR in cell cycle arrest and genome maintenance. *Genes Dev* 2003; **17**: 615-628
6 **Lee JH**, Paull TT. ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. *Science* 2005; **308**: 551-554
7 **Limoli CL**, Giedzinski E, Bonner WM, Cleaver JE. UV-induced replication arrest in the xeroderma pigmentosum variant leads to DNA double-strand breaks, gamma -H2AX formation, and Mre11 relocalization. *Proc Natl Acad Sci USA* 2002; **99**: 233-238
8 **Helt CE**, Cliby WA, Keng PC, Bambara RA, O'Reilly MA. Ataxia telangiectasia mutated (ATM) and ATM and Rad3-related protein exhibit selective target specificities in response to different forms of DNA damage. *J Biol Chem* 2005; **280**: 1186-1192
9 **Zhao H**, Piwnica-Worms H. ATR-mediated checkpoint pathways regulate phosphorylation and activation of human Chk1. *Mol Cell Biol* 2001; **21**: 4129-4139
10 **Guo Z**, Kumagai A, Wang SX, Dunphy WG. Requirement for Atr in phosphorylation of Chk1 and cell cycle regulation

in response to DNA replication blocks and UV-damaged DNA in Xenopus egg extracts. *Genes Dev* 2000; **14**: 2745-2756
11 **Liu Q**, Guntuku S, Cui XS, Matsuoka S, Cortez D, Tamai K, Luo G, Carattini-Rivera S, DeMayo F, Bradley A, Donehower LA, Elledge SJ. Chk1 is an essential kinase that is regulated by Atr and required for the G(2)/M DNA damage checkpoint. *Genes Dev* 2000; **14**: 1448-1459
12 **Bendjennat M**, Boulaire J, Jascur T, Brickner H, Barbier V, Sarasin A, Fotedar A, Fotedar R. UV irradiation triggers ubiquitin-dependent degradation of p21(WAF1) to promote DNA repair. *Cell* 2003; **114**: 599-610
13 **Uziel T**, Lerenthal Y, Moyal L, Andegeko Y, Mittelman L, Shiloh Y. Requirement of the MRN complex for ATM activation by DNA damage. *EMBO J* 2003; **22**: 5612-5621
14 **Carson CT**, Schwartz RA, Stracker TH, Lilley CE, Lee DV, Weitzman MD. The Mre11 complex is required for ATM activation and the G2/M checkpoint. *EMBO J* 2003; **22**: 6610-6620
15 **Stiff T**, Reis C, Alderton GK, Woodbine L, O'Driscoll M, Jeggo PA. Nbs1 is required for ATR-dependent phosphorylation events. *EMBO J* 2005; **24**: 199-208
16 **Kudoh A**, Fujita M, Zhang L, Shirata N, Daikoku T, Sugaya Y, Isomura H, Nishiyama Y, Tsurumi T. Epstein-Barr virus lytic replication elicits ATM checkpoint signal transduction while providing an S-phase-like cellular environment. *J Biol Chem* 2005; **280**: 8156-8163
17 **Daniel R**, Kao G, Taganov K, Greger JG, Favorova O, Merkel G, Yen TJ, Katz RA, Skalka AM. Evidence that the retroviral DNA integration process triggers an ATR-dependent DNA damage response. *Proc Natl Acad Sci USA* 2003; **100**: 4778-4783
18 **Zimmerman ES**, Sherman MP, Blackett JL, Neidleman JA, Kreis C, Mundt P, Williams SA, Warmerdam M, Kahn J, Hecht FM, Grant RM, de Noronha CM, Weyrich AS, Greene WC, Planelles V. Human immunodeficiency virus type 1 Vpr induces DNA replication stress in vitro and in vivo. *J Virol* 2006; **80**: 10407-10418
19 **Roshal M**, Kim B, Zhu Y, Nghiem P, Planelles V. Activation of the ATR-mediated DNA damage response by the HIV-1 viral protein R. *J Biol Chem* 2003; **278**: 25879-25886
20 **Lau A**, Swinbank KM, Ahmed PS, Taylor DL, Jackson SP, Smith GC, O'Connor MJ. Suppression of HIV-1 infection by a small molecule inhibitor of the ATM kinase. *Nat Cell Biol* 2005; **7**: 493-500
21 **Wilkinson DE**, Weller SK. Herpes simplex virus type I disrupts the ATR-dependent DNA-damage response during lytic infection. *J Cell Sci* 2006; **119**: 2695-2703
22 **Lilley CE**, Carson CT, Muotri AR, Gage FH, Weitzman MD. DNA repair proteins affect the lifecycle of herpes simplex virus 1. *Proc Natl Acad Sci USA* 2005; **102**: 5844-5849
23 **Gaspar M**, Shenk T. Human cytomegalovirus inhibits a DNA damage response by mislocalizing checkpoint proteins. *Proc Natl Acad Sci USA* 2006; **103**: 2821-2826
24 **Liu Y**, Shevchenko A, Shevchenko A, Berk AJ. Adenovirus exploits the cellular aggresome response to accelerate inactivation of the MRN complex. *J Virol* 2005; **79**: 14004-14016
25 **Stracker TH**, Carson CT, Weitzman MD. Adenovirus oncoproteins inactivate the Mre11-Rad50-NBS1 DNA repair complex. *Nature* 2002; **418**: 348-352
26 **Shin YC**, Nakamura H, Liang X, Feng P, Chang H, Kowalik TF, Jung JU. Inhibition of the ATM/p53 signal transduction pathway by Kaposi's sarcoma-associated herpesvirus interferon regulatory factor 1. *J Virol* 2006; **80**: 2257-2266
27 **Liang X**, Pickering MT, Cho NH, Chang H, Volkert MR, Kowalik TF, Jung JU. Deregulation of DNA damage signal transduction by herpesvirus latency-associated M2. *J Virol* 2006; **80**: 5862-5874
28 **Lin M**, Chen Q, Yang LY, Li WY, Cao XB, Wu JR, Peng YP, Chen MR. Hepatitis B virus infection and replication in primarily cultured human fetal hepatocytes. *World J Gastroenterol* 2007; **13**: 1027-1031
29 **Dimitrova DS**, Gilbert DM. Stability and nuclear distribution of mammalian replication protein A heterotrimeric complex. *Exp Cell Res* 2000; **254**: 321-327
30 **Nunnari G**, Argyris E, Fang J, Mehlman KE, Pomerantz RJ, Daniel R. Inhibition of HIV-1 replication by caffeine and caffeine-related methylxanthines. *Virology* 2005; **335**: 177-184
31 **Ward IM**, Chen J. Histone H2AX is phosphorylated in an ATR-dependent manner in response to replicational stress. *J Biol Chem* 2001; **276**: 47759-47762
32 **Weitzman MD**, Carson CT, Schwartz RA, Lilley CE. Interactions of viruses with the cellular DNA repair machinery. *DNA Repair* (Amst) 2004; **3**: 1165-1173
33 **Everett RD**. Interactions between DNA viruses, ND10 and the DNA damage response. *Cell Microbiol* 2006; **8**: 365-374
34 **Lilley CE**, Schwartz RA, Weitzman MD. Using or abusing: viruses and the cellular DNA damage response. *Trends Microbiol* 2007; **15**: 119-126
35 **Jurvansuu J**, Raj K, Stasiak A, Beard P. Viral transport of DNA damage that mimics a stalled replication fork. *J Virol* 2005; **79**: 569-580
36 **Hoeijmakers JH**. Genome maintenance mechanisms for preventing cancer. *Nature* 2001; **411**: 366-374
37 **Lee AT**, Ren J, Wong ET, Ban KH, Lee LA, Lee CG. The hepatitis B virus X protein sensitizes HepG2 cells to UV light-induced DNA damage. *J Biol Chem* 2005; **280**: 33525-33535
38 **Leupin O**, Bontron S, Schaeffer C, Strubin M. Hepatitis B virus X protein stimulates viral genome replication via a DDB1-dependent pathway distinct from that leading to cell death. *J Virol* 2005; **79**: 4238-4245
39 **White E**. Mechanisms of apoptosis regulation by viral oncogenes in infection and tumorigenesis. *Cell Death Differ* 2006; **13**: 1371-1377

S- Editor Zhong XY **L- Editor** Li M **E- Editor** Ma WH